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# Comparative Replicate Analyses of a Variety of Inoculated Fishery Products and Rehydrated Potato Flakes for *Escherichia coli* Using the Rapid Membrane Overlay Method and the Classical Most Probable Number Method

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# COMPARATIVE REPLICATE ANALYSES OF A VARIETY OF INOCULATED FISHERY PRODUCTS AND REHYDRATED POTATO FLAKES FOR *Escherichia coli* USING THE RAPID MEMBRANE OVERLAY METHOD AND THE CLASSICAL MOST PROBABLE NUMBER METHOD

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## TABLE OF CONTENTS


| | Page |
|---|---|
| ABSTRACT/RÉSUMÉ | iv |
| INTRODUCTION | 1 |
| MATERIALS AND METHODS | 1 |
| Inoculation of samples | 1 |
| Media and membranes | 2 |
| Enumeration procedure | 2 |
| Membrane overlay method (MOM) | 2 |
| Most probable number procedure | 2 |
| Standard plate count procedure | 3 |
| Experimental design | 3 |
| Statistical protocol | 3 |
| RESULTS | 4 |
| DISCUSSION | 4 |
| ACKNOWLEDGMENTS | 5 |
| REFERENCES | 6 |


## LIST OF TABLES


| Table | | Page |
|---|---|---|
| 1 | Geometric means of counts of E. coli per 100 g of food product obtained during 29 comparisons of methods | 7 |
| 2 | Results of statistical analysis on 29 comparisons of methods for determining E. coli. | 9 |


## LIST OF FIGURES


| Figure | | Page |
|---|---|---|
| 1 | Average count of E. coli obtained by the MOM compared with that obtained, in the same samples, by the MPN method in comparisons 1-9, Table 1 | 10 |
| 2. | Average count of E. coli obtained by the MOM compared with that obtained, in the same samples, by the MPN method in comparisons 10-19, Table 1 | 11 |
| 3 | Average count of E. coli obtained by the MOM compared with that obtained, in the same samples,by the SPC method in comparisons 10-19, Table 1 | 12 |
| 4 | Average log counts of E. coli obtained by the MPN compared with that obtained, in the same samples, by the SPC method in comparisons 10-19, Table 1 | 13 |
| 5 | Average log counts of E. coli obtained by the (DFO) compared with that obtained, in the same samples, by the HPB membrane overlay method in comparisons 20-24, Table 1 | 14 |
| 6 | Average log counts of E. coli obtained by the MOM compared with that obtained, in the same samples, by the MPN method in comparisons 25-29, Table 1 | 15 |
| 7 | Average log counts of E. coli obtained by the MOM compared with that obtained, in the same samples, by the MPN method in comparisons 1-19 and 25-29 | 16 |
| 8 | Average log counts of E. coli obtained by the MOM method compared with that obtained, in the same samples, by the MPN method in comparisons 1-19 and 25-29 | 17 |

## ABSTRACT

SMANDO, R. F., and C. SCHWARZ. 1991. Comparative replicate analyses of a variety of inoculated fishery products and rehydrated potato flakes for Escherichia coli using the rapid membrane overlay method and the classical most probable number method. Can. Tech. Rep. Fish. Aquat. Sci. 1768: iv + 17 p.

This study was undertaken to compare a relatively new technique, the rapid membrane overlay method (MOM), to the established, rather lengthy, most probable number (MPN) method, of enumerating Escherichia coli in inoculated fishery products and rehydrated mashed potato flakes. For some analyses, the standard plate count (SPC) method was used as a standard reference comparison system. Formal statistical analyses showed that the three methods were equally precise in enumerating E. coli in the inoculated fishery and potato products. At lower levels of E. coli there was slight variability between the two methods. This is of no major concern as it is anticipated that the membrane overlay methodology will be incorporated in the Department of Fisheries and Oceans bacteriological reference manual only as a screening method for E. coli in both raw and cooked fishery products. It is suggested here that if the Department of Fisheries and Oceans bacteriological guidelines for E. coli are exceeded, analysts should, the following day (10 hours later), proceed to reanalyze the same partially frozen product using the official 5:5:5 MPN series technique before the product can be officially rejected for marketing. The MOM technique is the preferred screening technique due to the rapidity and decreased cost of analyses.


Key words: rapid food analysis; sanitary indicator organisms; membrane overlay methodology.


## RÉSUMÉ

Smando, R.F., and C. SCHWARZ. 1991. Comparative replicate analyses of a variety of inoculated fishery products and rehydrated potato flakes for Escherichia coli using the rapid membrane overlay method and the classical most probable number method. Can. Tech. Rep. Fish. Aquat. Sci. 1768: iv + 17 p.

La présente étude avait pour but de comparer, d'une part, une technique relativement récent de dénombrement d'Escherichia coli dans divers produits de la mer et dans des floçons de pommes de terre réhydratés, la méthode rapide de nappage de la membrane, et d'autre part, la méthode bien connue du nombre le plus probable, qui demande plus de temps. Dans certains analyses, on a eu recours à la méthode de numération standard sur plaques (NSP) comme étalon. Les analyses statistiques ont montré que les trois méthodes permettaient de dénombrer E. coli avec un degré de précision égal dans les pommes de terre et les produits de la mer ensemencés. A des concentrations plus faibles d'E. coli, on a observé une légère variabilité entre les deux méthodes. Cette variabilité ne pose pas de difficulté, car on croit que la méthode ne sera pas adoptée par le Ministère des Pêches et des Océans, et inscrite dans le manuel de référence en bacteriologie, qu'à titre de méthode de dépistage d'E. coli dans les produits de la mer frais ou cuits. Dans les cas où la concentration d'E. coli excède les normes bactériologiques de Pêches et Océans, il est conseillé aux analystes de réanalyser le même produit partiellement congelé le jour suivant (18 heures plus tard) au moyen de la méthode NPP, série 5:5:5, avant de rejeter officiellement le produit. La méthode de nappage de la membrane constitue la méthode de dépistage préférée en raison de sa rapidité et de son faible coût.


Mots-clés: analyse rapide des aliments; organismes indicateurs; méthode de nappage de la membrane.

## INTRODUCTION

At present the official Department of Fisheries and Oceans method for enumerating the bacterium, Escherichia coli, in fishery products is the Most Probable Number (MPN) Method. This multi-tube method involves the inoculation of fifteen tube sets (5:5:5 MPN series) using relatively costly materials and requires a minimum of five working days to complete for E. coli confirmation. Therefore, there have been a number of efforts to determine a more rapid, less tedious and less costly methodology for estimating Escherichia coli using a more direct method than the MPN Method. Rapid analysis would benefit not only government laboratory personnel, but also importers of fish products who are confronted with high storage costs and fluctuating markets. One such rapid method is the hydrophobic grid membrane filter technique (Sharpe et al. 1981, 1983). Another method that shows much promise is the direct plating (DP) Agar technique of Delaney et al. (1962), developed initially for water analyses and later employed by Anderson and Baird-Parker (1975) to enumerate E. coli type I in food. Both methods were found to be reliable and precise and depend on stained indole (formed from the breakdown of the amino acid tryptophane) positive colonies following incubation of inoculated membranes for 18-24 hours at 44.5 $\pm$ 0.2$^{o}$C on tryptone bile agar (TBA).

Anderson and Baird-Parker (1975), found that 95.1% of the presumptive E. coli growing on membranes on TBA were identified as E. coli type I (IMViC ++--; Indole from the amino acid tryptophane; methyl red; Voges-Proskauer for the production of acetyl methyl carbinol; Citrate, utilized as only carbon source) and a further 3.4% as faecal coliforms on the basis of +++- and +---IMViC reactions (Geldreich 1966; Thatcher and Clark 1968). A few years later Rayman et al. (1979) showed that 96.6% of the indole positive isolates from the DP tryptone bile medium gave IMViC reactions characteristic of E. coli type I. Rayman et al. also commented that despite the inability of the DP method to enumerate E. coli type II and intermediate types which comprise 3-5% of the "Escherichia" strains (Ewing 1972), the method is preferable to the MPN method for enumerating E. coli in raw meats because of its lower variability, better recovery from frozen samples, rapidity, decreased requirement for media, and decreased costs for analysts' time.

In a later collaborative study by five Health Protection Branch (HPB) laboratories, Sharpe et al. (1983) compared the MPN, DP, and a hydrophobic grid membrane filter method. For ground beef, Parmesan cheese and cut green beans, the hydrophobic grid membrane filter method gave the highest recovery, although it was not statistically significant above that from the DP Method. Both of these filtration methods gave significantly higher recoveries than the MPN procedure, and for most foods either filter method was preferable over the MPN Method.

This present study utilized basically the same procedure as that used by Anderson and Baird-Parker (1975) and Health Protection Branch laboratories, with a slight modification to increase sensitivity. In an International Commission on Microbiological Specifications for Foods study (Rayman et al. 1979), participants plated out duplicate 1.0 mL volumes of the 1:5 and 1:10 dilutions of solid samples; one red stained colony in one of two duplicate membrane represented 500 and 1 000 confirmed E. coli per 100 g of food, respectively. In the Department of Fisheries and Oceans (DFO), fishery products are rejected for market if E. coli levels exceed 400 cells/100 g in three out of five raw sample units analyzed, or two out of five cooked sample units analyzed, and/or if one sample unit in either case exceeds 4 000 cells/100 g the disposition of the product based on the analyses of five sample units. Since DFO's lower rejection guideline is at 400 cells/100 g, the sensitivity of the DP Technique was modified so that one colony on one DP plate would represent 100 E. coli cells/100 g in a product.

The purpose of this investigation is to compare the accuracy and precision of measuring the amount of E. coli present in seafood and potato products. The comparison is made on the membrane overlay, most probable number, and standard plate count (SPC) methodologies at various levels of E. coli in inoculated samples analyzed. Of particular interest are the data slightly below or above the 400 cells/100 g DFO E. coli rejection level.

## MATERIALS AND METHODS

### INOCULATION OF SAMPLES

This study used five laboratory strains of E. coli isolated from scallops, shell-on shrimp, breaded cod fillets, peeled and deveined shrimp, lobster meat and other fishery products, and one strain of American Type Culture Collection (ATCC) culture 25922. These products were ino-

culated by submerging tissue housed in a wire mesh basket, into a 4 L polyethylene bucket containing vigorously stirring preinoculated 0.1% peptone water (chilled to 4°C). The basket with inoculated product was then lifted out of the inoculated suspension of cells, the excess suspension was allowed to drain from the product, the basket placed on tin foil, and the product sampled for analyses.

To inoculate dehydrated mashed potato with E. coli, 140 g of sterile potato flakes were added to 1 000 mL of diluted E. coli culture in 0.1% peptone water and allowed to rehydrate with vigorous mixing. One hundred gram analytical samples were then withdrawn from the larger inoculated sample for replicate analyses. All equipment and materials used in the inoculation procedure were sterilized either by autoclaving or by surface decontamination with Virocidin-X.

## MEDIA AND MEMBRANES

All media and membranes used in the membrane overlay method (MOM) were purchased from Oxoid Canada Ltd. For the Most Probable Number (MPN) and Standard Plate Count (SPC) analysis, media from Difco Laboratories Ltd. were used. Kovac's reagent used for staining of membranes was purchased from Roche Diagnostics.

## ENUMERATION PROCEDURE

Simultaneous reading by two analysts of gas positive tubes (inverted fermentation vials) and/or effervescence in the MPN procedure and counting of red colonies in the direct plating procedure were frequently carried out to minimize enumeration errors.

### Membrane Overlay Method (MOM)

Anderson and Baird-Parker's Modified Direct Plating Method: The direct plating method of Anderson and Baird-Parker (1975) was followed with modifications to increase the sensitivity of the procedure. Instead of 1:5 and 1:10 sample dilutions, a 1:2 sample dilution was made, with 0.1% peptone water (100 g product, 100 g diluent) in a Stomacher bag. The contents of the bag were then blended for one minute in a Stomacher lab blender 400 according to the method of Sharpe and Jackson (1972) and the homogenate filtered immediately through four layers of sterile cheesecloth placed on top of a 1 000 mL glass beaker which contained another Stomacher bag to collect the filtrate. After rapid filtration, 2.0 mL of the filtrate were placed on membranes on each of two duplicate pre-dried plates. The inoculum was spread evenly with a spreader bar, and the plates with their lids ajar were placed in a 35°C incubator for up to one-half hour to facilitate further drying and absorption of inocula into the TBA agar medium. After the inoculum was absorbed and the lids secured, the plates were inverted, placed in an airtight plastic container and floated on the surface of a water bath incubator set at 44.5 ± 0.2°C. The plates (not more than two deep) were incubated for a maximum of 18 hours.

Duplicate membranes were transferred by flat forceps onto the surface of 9.0 cm Whatman filter papers saturated with 2.0 mL of Kovac's reagent. The filter papers were then placed inside the lids of the duplicate plates. Indole positive red colonies (directly counted) were obvious in 10-15 seconds. At first, red colorations of indole positive colonies were quite diffuse around each colony. After further staining for 10 minutes and drying of membranes on paper towels in a fumehood, the indole positive halos around the colonies disappeared leaving only separate and discrete red stained colonies which were easily counted, even if mixtures of indole negative colonies were present. This reddish colour was bleached to a greenish colour after a couple of hours. One red positive colony on one membrane represents 100 confirmed E. coli per 100 g sample.

Health Protection Branch Direct Plating Methodology: The DP method was used according to HPB's specifications which can be obtained from Microbial Hazards Bureau, Frederick G. Banting Building, Ross Avenue, Ottawa, Ontario K1A 0L2. Essentially, 10 g of rehydrated mashed potato flakes were weighed out and diluted 1:5 with sterile peptone water and 1.0 mL volumes were plated out on membranes resting on duplicate TBA plates. One red indole positive colony per plate represented 500 confirmed E. coli per 100 g sample unit analyzed.

### Most Probable Number Procedure

This procedure was used according to the Department of Fisheries and Oceans' standard reference manual, Standard Procedures for Bacteriological Analysis (1988). The 5:5:5 MPN series was used which calls for 10 ml, 1.0 ml and 0.1

mL inoculations into lauryl tryptose broth (LTB) tubes. The DFO procedure was modified by using 1.0 mL of a 1:100 dilution in place of 0.1 mL aliquots to eliminate foam problems resulting from blender homogenization.

Standard Plate Count Procedure

Standard Plate Count (SPC) data were generated according to DFO's standard reference manual using the blender for homogenization. Routine 1:10 dilutions were made to cover the range of SPC counts.

Standard Plate Count (SPC) data, along with the DP data were generated from the Stomacher homogenate. Two mL of the filtered 1:2 homogenate were pipetted onto duplicate SPC and DP plates. Early plating studies showed that both the blender and Stomacher were similar in releasing organisms from products. The Stomacher was routinely used for the DP Method because the homogenate was not as viscous and thus filtration through cheesecloth was easier.

Standard Plate Count data was used as an absolute comparision reference system.

Experimental design

The experimental design used in the assays is a sub-sampling design (Steel and Torrie 1980, p. 153-171; Sokol and Rohlf 1981, p. 271-320. Since the number of samples differs among procedures, and the number of sub-samples differs among methods (two for MOM and SPC; one for MPN), the design is unbalanced, and the analysis is more complicated than in balanced designs but readily accomplished using the SAS system for statistical analysis (SAS 1985).

STATISTICAL PROTOCOL

A total of 29 preparations of seafood or potato products were inoculated with E. coli and analyzed by two or more methods. The comparison of the different methods of determining E. coli in these products is presented in the following ways:

i) When DFO's MOM was compared to MPN, three to 10 analytical 100 g samples were selected from each preparation. Two sub-samples from each analytical sample were analyzed using MOM and one sub-sample from each sample was analyzed using MPN.

ii) When DFO's MOM was compared to MPN and SPC, five to 10 analytical samples were selected from each preparation. Two sub-samples from each analytical sample were analyzed using MOM, two sub-samples from each analytical sample were analyzed using SPC, and one sub-sample from each sample was analyzed using MPN.

iii) When MOM in the Department of Fisheries and Oceans (DFO) was compared to MOM in the Health Protection Branch (HPB), five analytical samples were selected from each preparation. Two sub-samples from each analytical sample were analyzed.

iv) When MOM was compared to MPN, the Stomacher was used in the preparation of samples. Five analytical samples were selected from each preparation. Two sub-samples from each analytical sample were analyzed using MOM; one sub-sample from each analytical sample was analyzed using MPN.

Prior to any formal analysis, the data was screened by plots of the standard deviation vs the mean and by box plots to identify any anomalous values. No unusual values were detected and all values were included in the statistical analysis.

The logarithm to the base 10 of the E. coli numbers was used in all analyses. This transformation provided the following benefits:

i) It stabilized the variance of the sub-sample results so that the variation among the logarithm of the sub-sample readings was independent of the mean. This is often a necessary assumption for many statistical procedures.

ii) It reduced the influence of extreme but otherwise valid data values. An average of the logarithms of values is equivalent to the logarithm of the geometric mean of the original data values which is well known to be less sensitive to extreme values than the usual arithmetic mean.

iii) It reduced the range of the data values from 300-16 000 counts to between two and five on the logarithm scale.

## RESULTS

Geometric means of E. coli counts obtained by MOM, MPN, SPC, HPB methods in 10 fish food products ranged from 110 to 11 000 per 100 g (Table 1).

A summary of the statistical analyses of paired comparisons of methods reveal that there were no overall significant differences between the four methodologies tested (Table 2). If two methods gave exactly the same results, the estimated ratio of the counts between the two methods should be close to one. Ratios much less than one, or ratios much greater than one, indicate that one method gives, on average, a higher or lower reading than the other method. A 95% confidence interval for the ratio of readings between the two methods gives a range of plausible values for the ratio of readings between the two methods. [The confidence interval is asymmetric about the estimated ratio since the confidence interval was found on the logarithm of the values and then anti-logarithms were taken]. If the two methods gave, on average, different readings, the 95% confidence interval would likely not contain the value 1.00. The p-value reported in Table 2 is a measure of the evidence against equal readings. Small p-values (less than 0.05) indicate good evidence that the ratio of readings is not 1.00; large p-values (greater than 0.15) indicate there is little evidence that the ratio of the readings is not 1.0; intermediate p-values (between 0.05 and 0.15) indicate weak evidence that the ratio is not 1.0.

The comparison of MPN vs SPC (Comparisons 10-19) reveal that the ratio of counts is 0.88 (or 88%); comparisons 20-24 and 25-29 show MOM readings slightly higher than HPB (membrane overlay method), and MPN methods tested, by 129% and 141%, respectively. This data must be interpreted with care because it is at the lower readings (around the Department of Fisheries and Oceans' rejection level of 400 E. coli cells per 100 g of sample analysed) where the quantitative counts are not as precise as one would like. This is due to the limiting sensitivity of the methods compared at these lower counts, even though the detectability of E. coli by the MOM procedure is excellent, at these low E. coli values. The sensitivity of the methods is as follows: one colony on the DFO-MOM agar plate represents 100 confirmed E. coli cells per 100 g sample analyzed. The SPC method has similar sensitivity. In the HPB membrane overlay method, one colony on the agar plate represents 500 confirmed E. coli cells per 100 g sample. Theoretically, to accurately enumerate E. coli by these direct plate methods, counts on plates should range from 20-200/plate. If, for example, a sample contains E. coli numbers at 400/100 g, a MOM agar plate would contain only four colonies, and with the HPB method, the plate would contain no colonies (<500/100 g E. coli).

The mean logarithms of counts of one method for each sample versus the logarithms of counts of the other method compared are shown in Fig. 1-8. If no differences existed between two methods compared, the points in each figure should be aligned along the reference line and be equally scattered above and below the line. Only Fig. 4, 5 and 6 (Comparisons 10-29) show some scatter of points due to lower counts enumerated, as previously described. Figure 7 presents a combined plot overlaying Fig. 1, 2 and 6 (Comparisons 1-19 and 25-29) for comparison of MOM to MPN. One point appears for each sample from each preparation. In Fig. 8, only the average within preparations is plotted. From these two figures alone, it can be seen there is little difference between the readings from MOM to MPN except at the lower counts where MOM often gives higher readings than MPN. These data (Fig. 2, 6, 7) occur in Comparisons 14-19 and 25-29, Table 1. Also, statistical analysis of Comparisons 1 to 13 representing fishery products reveals there is no overall difference between the MOM and MPN methods since the estimated (non-significant) ratio of the MOM reading is 97% of the MPN reading (Table 2). The 95% confidence interval for the true ratio is 0.88 to 1.06 which indicates that the true ratio could plausibly lie between 88% and 106% (note that there are no low values in these tables; colony counts are also in the range, 20-200 per plate).

## DISCUSSION

The similarity of accuracy and precision of replicate counts between the Membrane Overlay (MOM) and Most Probable Number Method (MPN) suggests that both methodologies were of equal value in enumerating Escherichia coli in fishery samples and rehydrated potato flakes inoculated with various levels of this bacterial species. The official method of the Department of Fisheries and Oceans for passing or rejecting fishery products for E. coli is the MPN method. Since the MOM yields similar counts to the MPN method, and since the sensitivity of MOM method is 100 confirmed E. coli per 100 g, and both methods

are identical to the standard plate count (SPC) methodology, it is recommended that the MOM method should be used on a routine basis to screen domestic and imported products for E. coli.

When the MOM is used on a routine basis as a screening method to potentially reject a fishery product due to E. coli, this method would correspond to single TBA plates containing greater than four indole positive red colonies (>400 E. coli per 100 g) in three out of five raw fishery sample units analyzed, or in two out of five cooked sample units analyzed. If values in this range were encountered, it is suggested that one day after the MOM results are known, the products be reanalyzed for enumerative E. coli using the fficial 5:5:5 MPN series. A resampling problem of reduced bacterial numbers due to refreezing and rethawing a product for subsequent MPN analyses would not take place, since products initially sampled for MOM analyses are partially in the frozen state.

The use of the MOM method in routine analysis results in much savings of time and costs. For example, to analyze one fishery product, the MOM technique would take only minutes, whereas to use the MPN method, hours would be required for the enumerative confirmation of E. coli via its multi-step procedures. The cost for the technique would only be a few dollars, whereas the cost of the MPN procedure could be 10 times more. From past experience the MOM method is also the preferred method due to its capability of supporting growth of a variety of E. coli strains tested on the TBA medium. From a previous comparative study of a variety of water samples using a membrane filtration technique versus MPN (Smando 1983), it was apparent that coliforms in general preferred growth on a selective solid agar medium. In the MPN method organisms are growing together in the same tubes and there is much competition for nutrients. In the MOM method a more selective medium (TBA) and temperature (44.5$^{O}$C) is used to enhance the growth of E. coli on this medium and to suppress the growth of other organisms that are of no public health significance. Individual cells on TBA grow separately from neighbouring cells into independent visible colonies without competition between neighbouring colonies. The MOM method was the preferred method used in the Edmonton Fisheries and Oceans' laboratory study (unpublished data/personal communication) where it was used only as a screening method. In most cases there were either no colonies growing on the TBA agar, or colonies growing on the agar stained negative in 18 hrs. Resampling of products and reanalyses of products via the 5:5:5 MPN series showed that the MOM method was, in fact, very sensitive in detecting E. coli, as subsequent MPN analyses often resulted in nil or low detectability. The MOM method thus appeared much more sensitive than the MPN method. There was no case where the MOM method would have passed a product which would have been rejected by the MPN method.

Along with time and cost savings in the laboratory, another important reason for using the MOM method over the MPN method on a routine basis for E. coli analysis on both domestic and import products is that detained products containing levels of E. coli below our bacteriological guidelines can be released for sale in 18 hours after samples are received in the laboratory for analysis. For the MPN technique, as many as three to five days are needed to run a multiple of steps for final results, a process that often involves overtime work during weekends and holidays.

In conclusion, the advantages of the accuracy, precision, reliability and low operating costs of the MOM method far outweigh the MPN method's good features. It is recommended that the MOM method be incorporated in Fisheries and Oceans bacteriological procedure manual for routine use in screening out E. coli in both domestic and import fishery products.

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Table 1. Geometric means of counts of *E. coli* per 100 g of food product obtained during 29 comparisons of methods (logarithms and number of replicates are given in parenthesis).

| COMPARISON | FOOD PRODUCT | MOM (DFO) | MPN | SPC | HPB |
|---|---|---|---|---|---|
| 1 | Scallops | 1300(3.1153,6) | 1500(3.1740,6) | - | - |
| 2 | R/H Shrimp | 4700(3.6679,5) | 4800(3.6792,6) | - | - |
| 3 | R/H Shrimp | 980(2.9933,5) | 1100(3.0307,5) | - | - |
| 4 | Breaded cod fillets | 4500(3.6487,10) | 5900(3.7718,10) | - | - |
| 5 | Breaded cod fillets | 7000(3.8444,7) | 7300(3.8646,7) | - | - |
| 6 | Small cooked shrimp | 6100(3.7853,7) | 5100(3.7040,7) | - | - |
| 7 | Cuttlefish balls | 2500(3.3978,10) | 2900(3.4622,10) | - | - |
| 8 | Breaded cod | 3600(3.5606,3) | 4000(3.6069,3) | - | - |
| 9 | Battered cod | 2800(3.4501,10) | 3000(3.4834,10) | - | - |
| 10 | R/H Shrimp | 3200(3.5017,10) | 2900(3.4698,10) | 3500(3.5480,10) | - |
| 11 | Scallops | 10000(4.0199,10) | 7900(3.8960,10) | 11000(4.0372,10) | - |
| 12 | P & D Shrimp | 4000(3.6073,10) | 3700(3.5703,10) | 4000(3.6042,10) | - |
| 13 | Lobster meat | 2100(3.3236,10) | 2500(3.4048,10) | 1900(3.2881,10) | - |
| 14 | Mashed potato flakes | 180(2.2590,5) | 180(2.2605,5) | 200(2.2937,5) | - |
| 15 | Mashed potato flakes | 300(2.4962,8) | 150(2.1798,8) | 330(2.5166,8) | - |
| 16 | Mashed potato flakes | 290(2.4635,5) | 340(2.5325,5) | 270(2.4334,5) | - |

Table 1. Cont'd.

| COMPARISON | FOOD PRODUCT | MOM (DFO) | MPN | SPC | HPB |
|---|---|---|---|---|---|
| 17 | Mashed potato flakes | 630(2.7963,5) | 740(2.8707,5) | 670(2.8287,5) | - |
| 18 | Mashed potato flakes | 230(2.3539,5) | 110(2.0578,5) | 310(2.4954,5) | - |
| 19 | Mashed potato flakes | 430(2.6299,5) | 590(2.7672,5) | 350(2.5487,5) | - |
| 20 | Mashed potato flakes | 5900(3.7732,5) | - | - | 3900(3.5872,5) |
| 21 | Mashed potato flakes | 4600(3.6606,5) | - | - | 4600(3.6633,5) |
| 22 | Mashed potato flakes | 11000(4.0408,5) | - | - | 9100(3.9569,5) |
| 23 | Mashed potato flakes | 800(2.9006,5) | - | - | <500(2.7592,5) |
| 24 | Mashed potato flakes | 2300(3.3567,5) | - | - | 2100(3.3151,5) |
| 25 | Mashed potato flakes | 580(2.7636,5) | 470(2.6693,5) | | - |
| 26 | Mashed potato flakes | 410(2.6141,5) | 290(2.4614,5) | - | - (used stomacher - for both techniques) |
| 27 | Mashed potato flakes | 890(2.9508,5) | 490(2.6859,5) | - | - |
| 28 | Mashed potato flakes | 1200(3.0925,5) | 1300(3.1246,5) | - | - |
| 29 | Mashed potato flakes | 760(2.8780,5) | 460(2.6630,5) | - | - |

Table 2. Results of statistical analysis on 29 comparisons of methods for determining E. coli. Means counts are summarized in Table 1.

| | | Results | | | |
|---|---|---|---|---|---|
| Figure | Comparison | p-value | Ratio | 95% Confidence Interval | Remarks |
| 1 | MOM vs MPN (1-9, Table 1) | .10 | .91 | (.82-1.02) | |
| 2 | MOM vs MPN (10-19, Table 1) | .17 | 1.08 | (.96-1.23) | |
| 3 | MOM vs SPC (10-19, Table 1) | .36 | 1.05 | (.95-1.16) | |
| 4 | MPN vs SPC (10-19, Table 1) | .04 | .88 | (.78-.99) | No significant differences were found if values below 2.0 were deleted. |
| 5 | MOM vs HPB (20-24, Table 1) | .002 | 1.29 | (1.13-1.47) | |
| 6 | MOM vs MPN (25-29, Table 1) | .011 | 1.41 | (1.24-1.60) | |
| 7, 8 | MOM vs MPN (1-19, 25-29, Table 1) | | | | |
| (combined) | MOM vs MPN (1-13, Table 1) | .39 | .97 | (.88, 1.06) | |

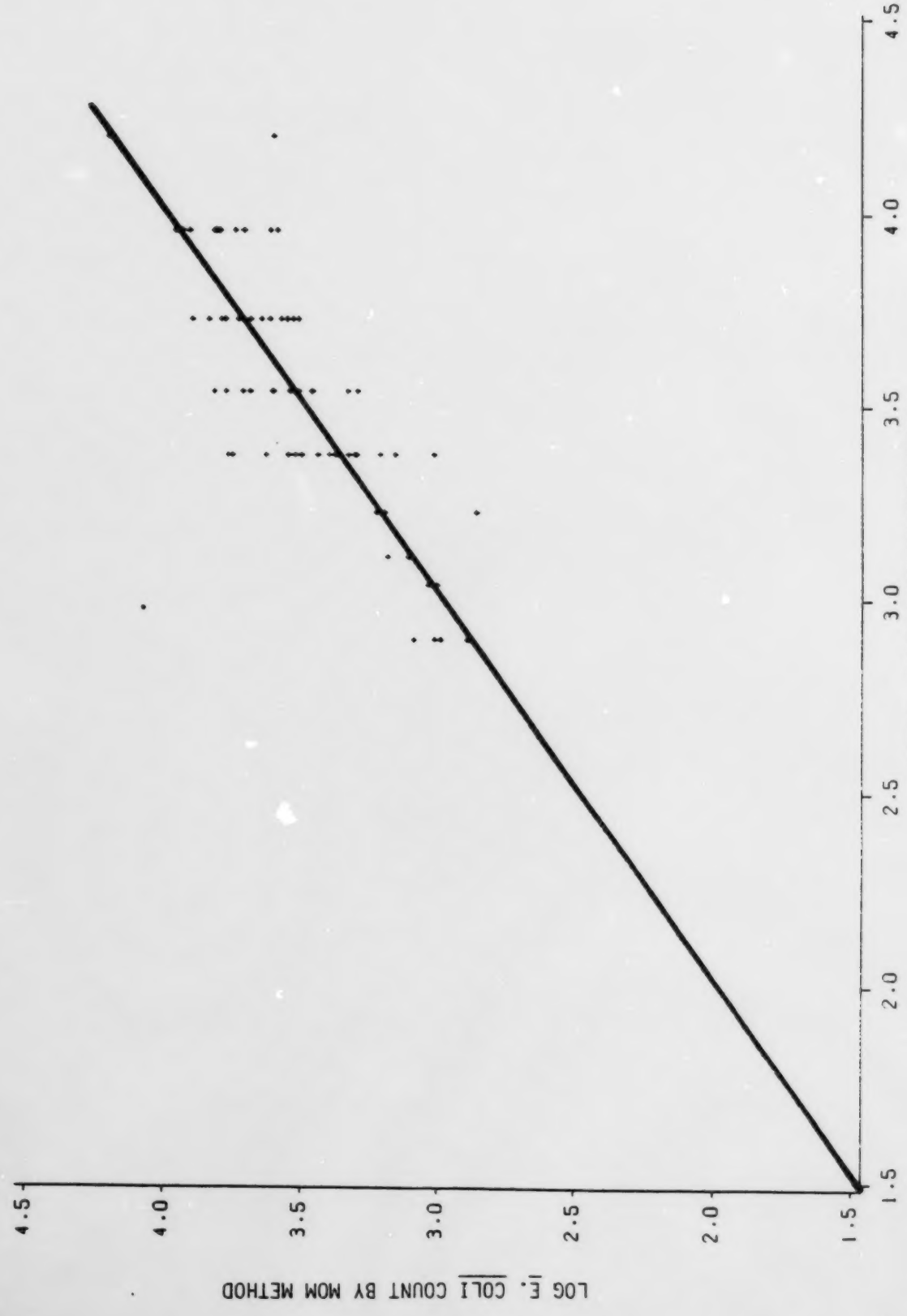


Fig. 1. Average count of E. coli obtained by the MOM compared with that obtained, in the same samples, by the MPN method in comparisons 1-9, Table 1. The line connects points of no difference.

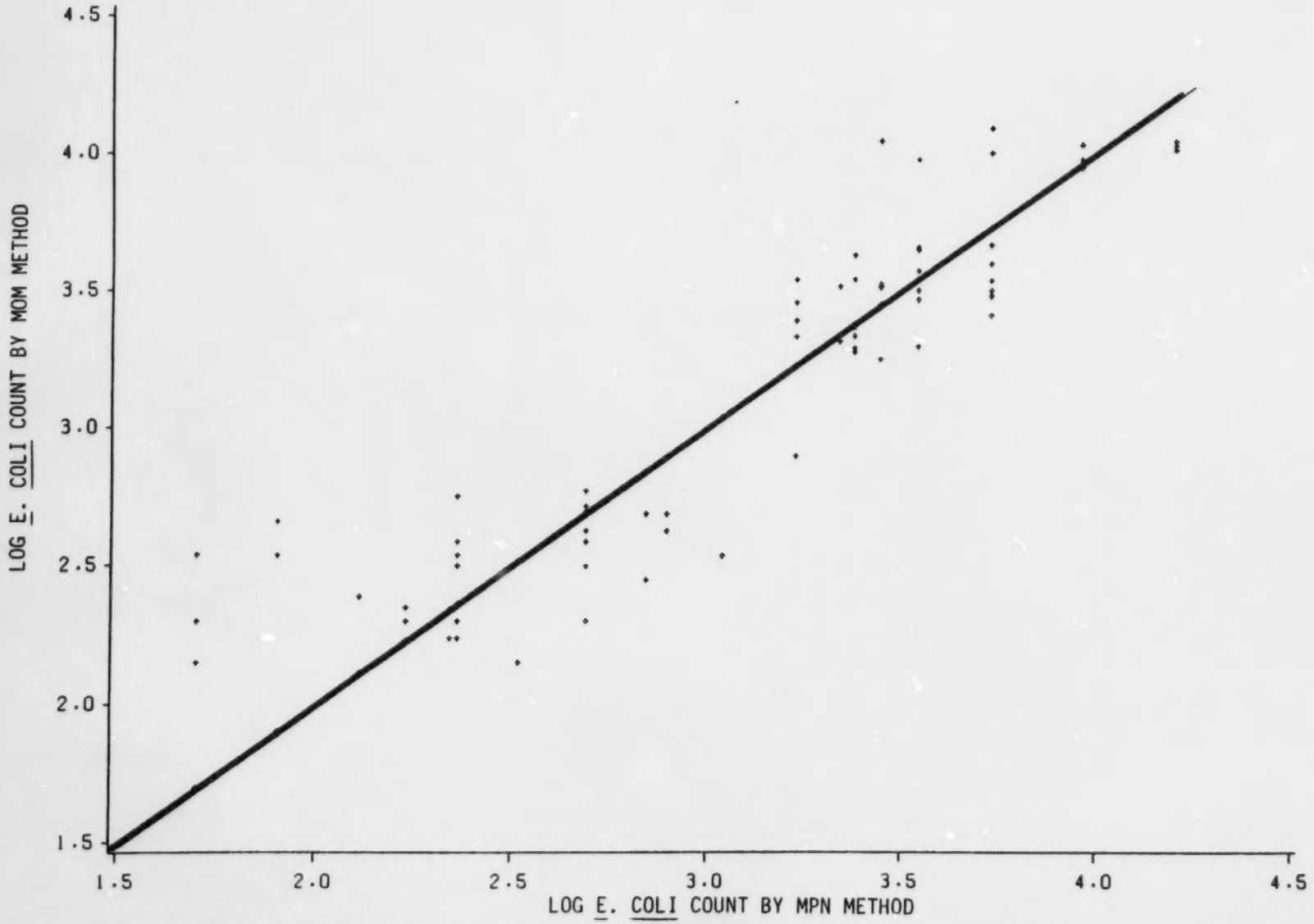


Fig. 2. Average count of E. coli obtained by the MOM compared with that obtained, in the same samples, by the MPN method in comparisons 10-19, Table 1. The line connects points of no difference.

Fig. 3. Average count of E. coli obtained by the MOM compared with that obtained, in the same samples, by the SPC method in comparisons 10-19, Table 1. The line connects points of no difference.

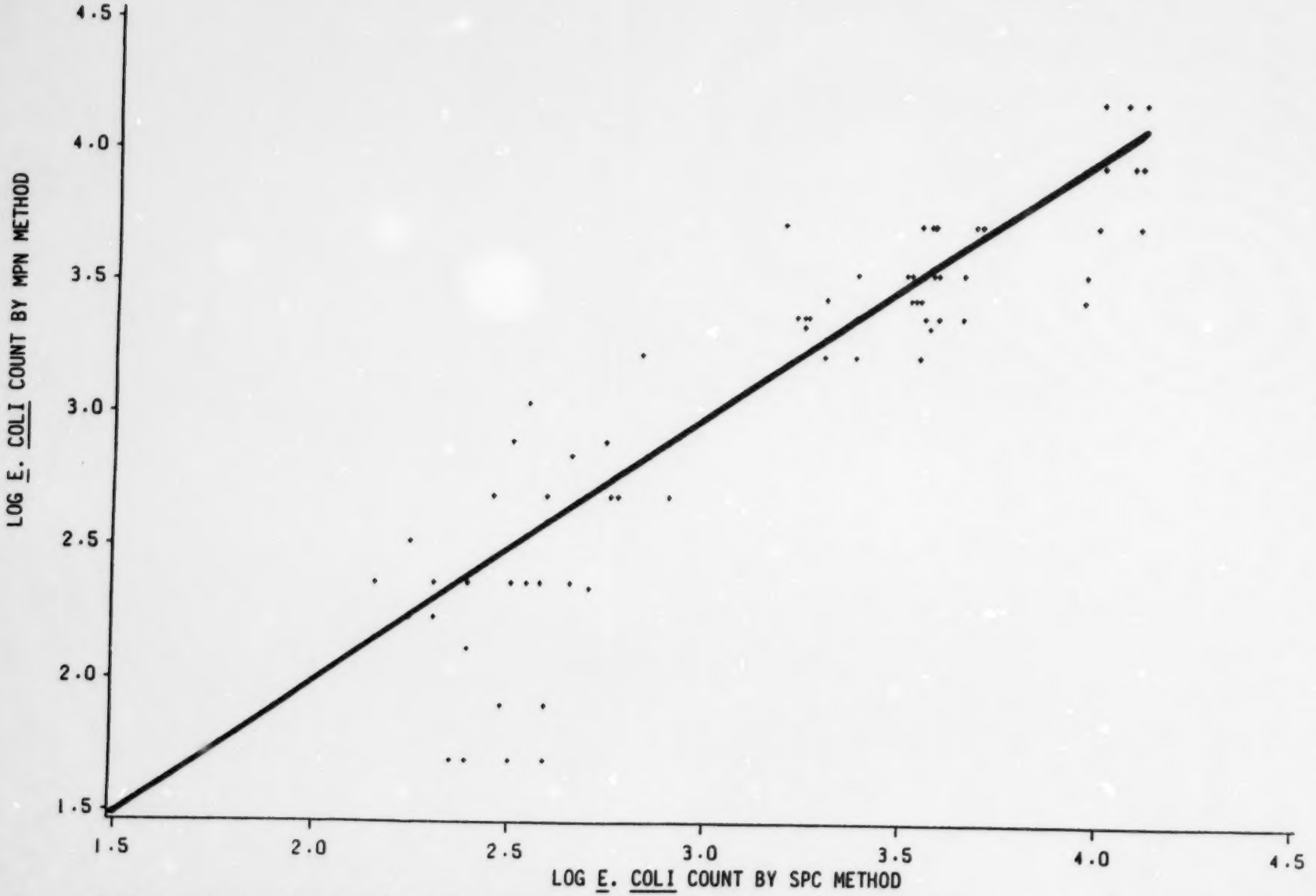


Fig. 4. Average log counts of E. coli obtained by the MPN compared with that obtained, in the same samples, by the SPC method in comparisons 10-19, Table 1. The line connects points of no difference.

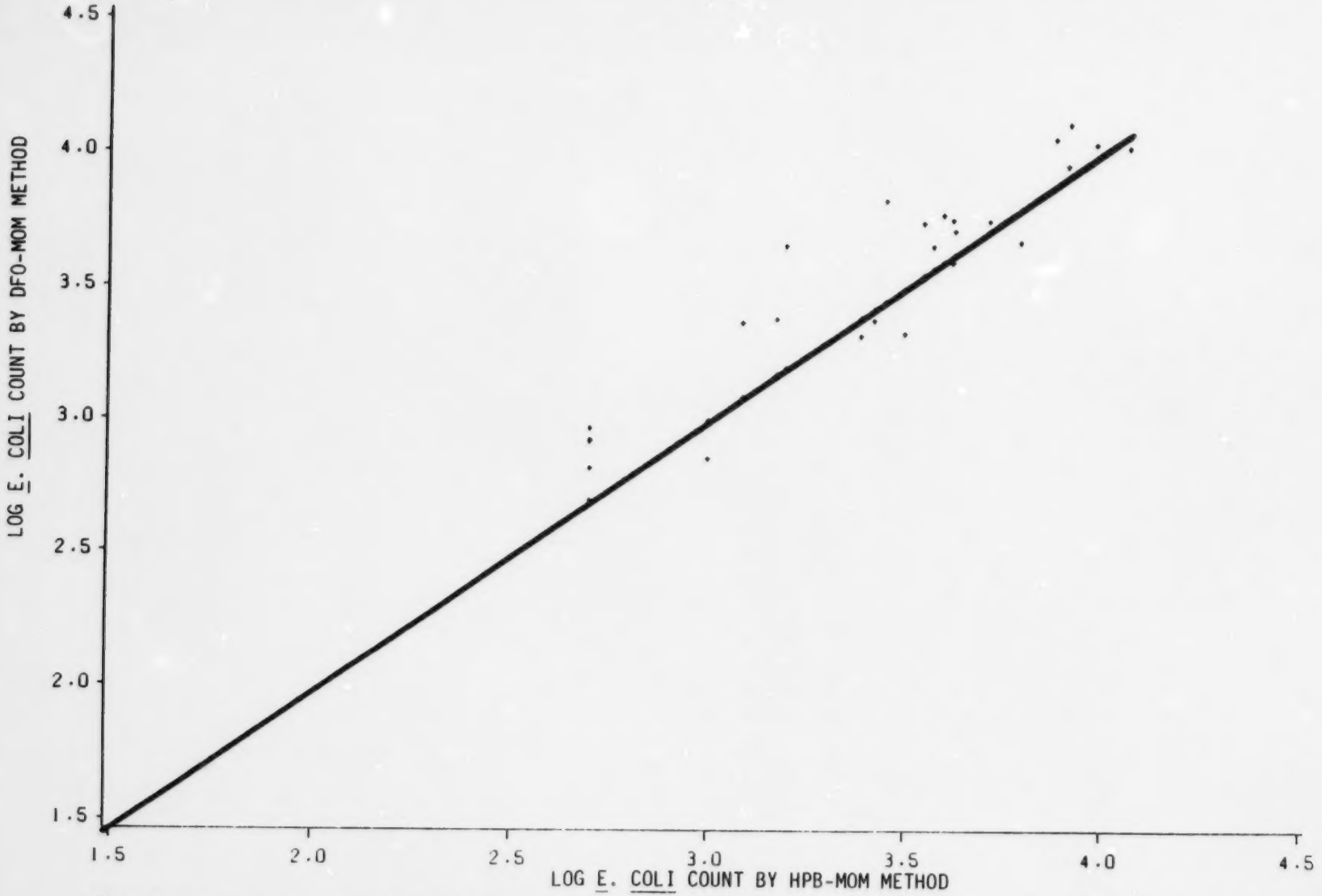


Fig. 5. Average log counts of E. coli obtained by the MOM (DFO) compared with that obtained, in the same samples, by the HPB membrane overlay method in comparisons 20-24, Table 1. The line connects points of no difference.

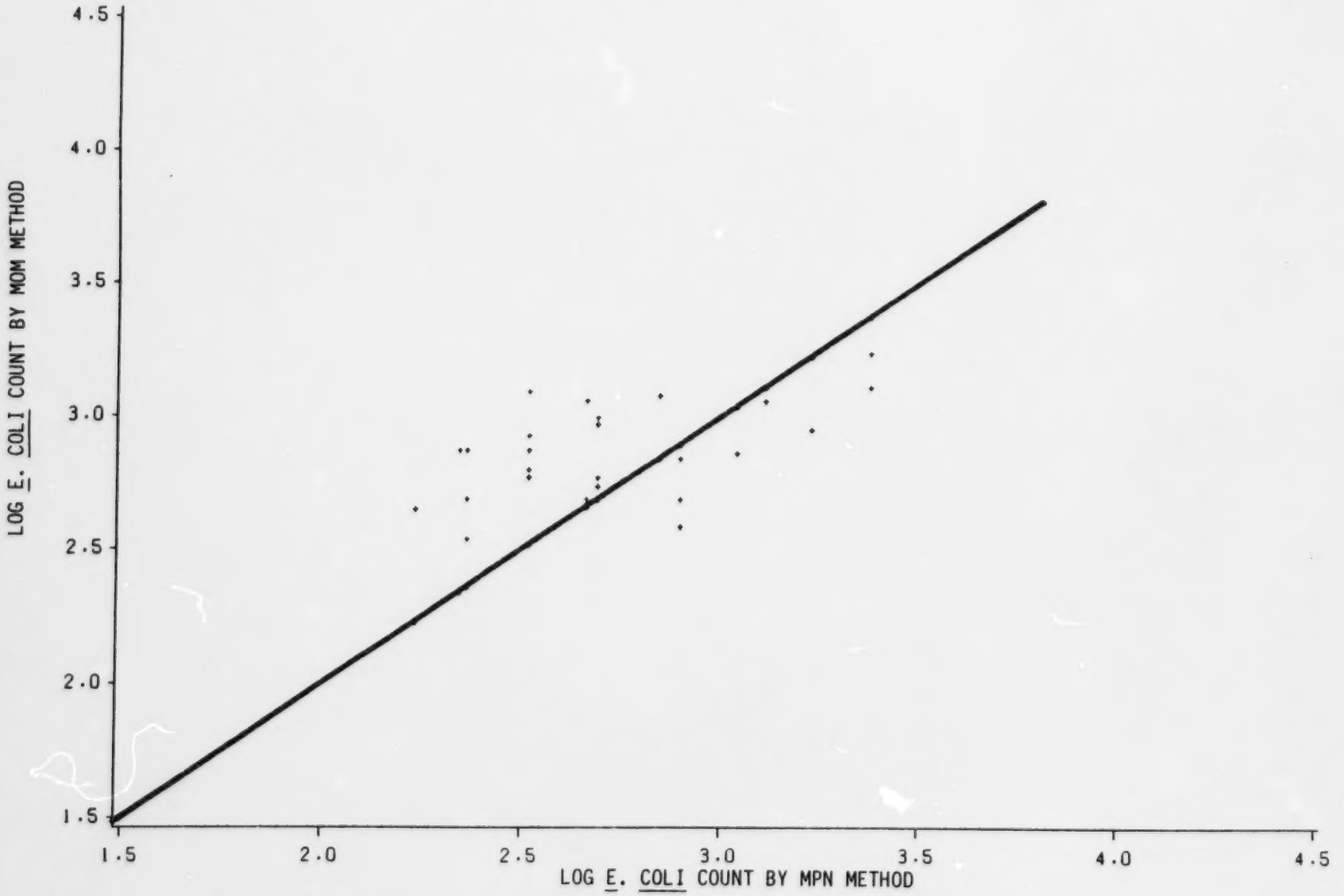


Fig. 6. Average log counts of E. coli obtained by the MOM compared with that obtained, in the same samples, by the MPN method in comparisons 25-29, Table 1. The line connects points of no difference.

Fig. 7. Average log counts of E. coli obtained by the MOM compared with that obtained, in the same samples, by the MPN method in comparisons 1-19 and 25-29. The line connects points of no difference.

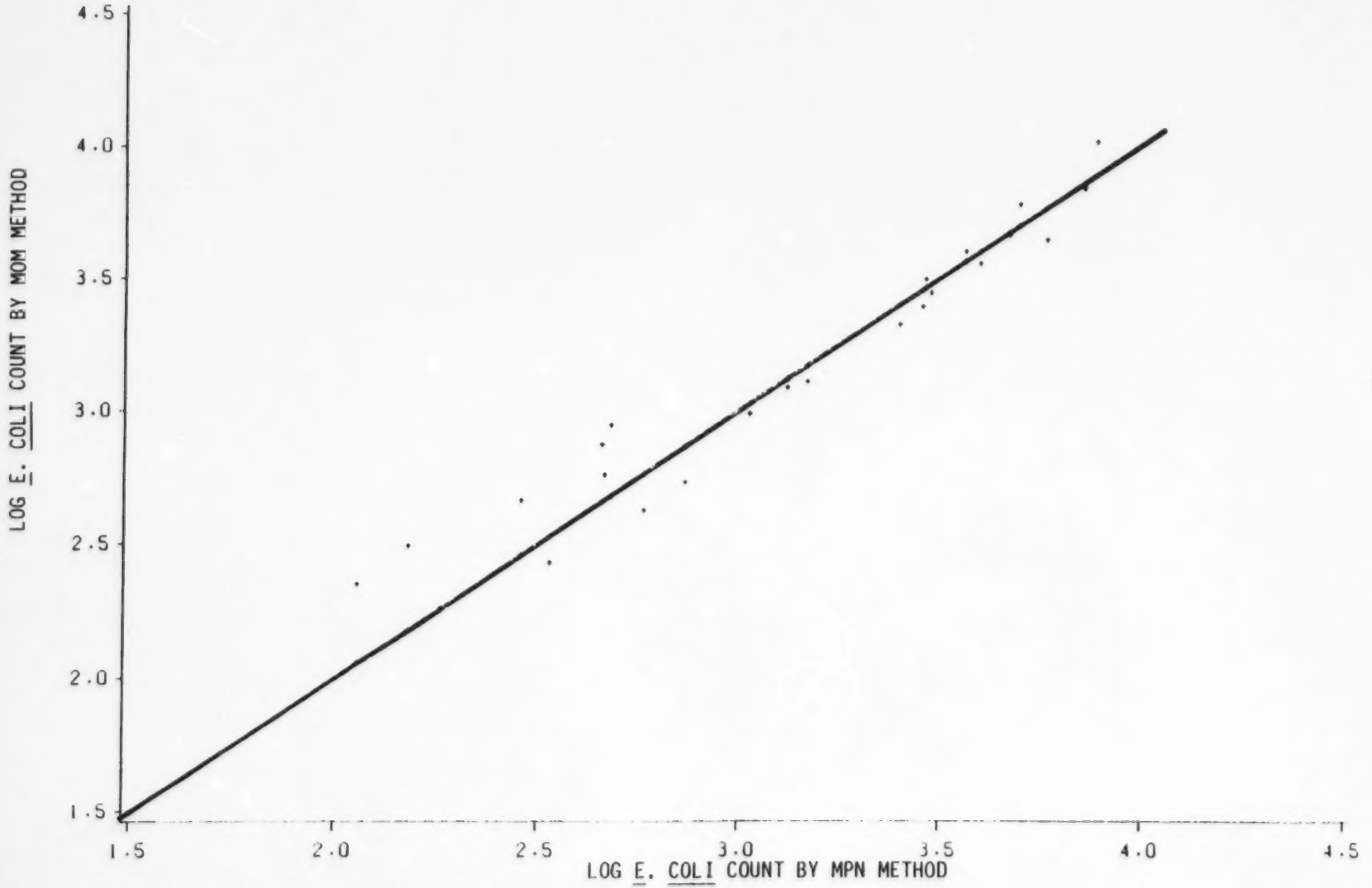


Fig. 8. Average log counts of E. coli obtained by the MOM method compared with that obtained, in the same samples, by the MPN method in comparisons 1-19 and 25-29. The line connects points of no difference.